

January 17, 1966

Miss Winnie M. Morgan  
Technical Reports Officer  
Office of Grants & Research Contracts  
Office of Space Science & Applications  
Washington, D.C. 20546

Re: STATUS REPORT: NASA PROJECT  
SC NASr-221/06-05-001  
7th Quarterly Report  
Oct. 1st to Dec. 31, 1965

1. The printer's proof of the paper entitled "Oxyhemoglobin Dissociation Curve in the Blood of Lungfish" was received, corrected and returned to the American Journal of Physiology. It is expected that this paper should appear in print within four to six months.

2. It was found that although it was possible with the equipment we had to measure oxygen consumption of the living fish when the aquarium were full of water, it proved impractical when the oxygen consumption was markedly lowered by aestivation and the volume of air in the system was markedly increased. Therefore, this attempt has been abandoned. The measurement was not vital to the success of our project, but would have provided interesting observations.

3. A very exciting lead has been achieved in the demonstration of an active principle in the brain of the aestivating lungfish

a) Dr. Dalton Jenkins of the University of Colorado has established extraction techniques, based on the work of Axelrod and others, for the extraction of hormones from the mid-brain of the lungfish. It is estimated that such a principle will most likely be polypeptide, but this is not known.

b) As reported we had four large lungfish in our aquarium. Two of these were forced into aestivation and in the third week of aestivation attempt was made to kill the fish without a struggle and to extract the brain immediately

c) Fish A in this series was about a two pound fish. He was found encysted and in a state of typical aestivation in the mud in the aquarium. Attempt was made to kill him rapidly but this was difficult with the use of the rib shears which we attempted to use on this occasion. So that this fish struggled for a considerable degree before we were able to sever his head from the body. Extracts of the brain were prepared and injected intervenously into a white rat. The CO<sub>2</sub> production was measured and there was no observable significant decrease in the CO<sub>2</sub> production during the next 24 hrs., nor was there a fall in the body temperature.

d) Fish B in this series weighed approximately two and one-half to three pounds and was found in the third week of aestivation to be well encysted and apparently in typical aestivation. On disturbing him he began to become active rapidly but we were able to transfer him to a large board and with a meat cleaver severed his head from the body in a single stroke. Thus, there was much less struggling with Fish B than with the previous one. Extracts were prepared from his brain which was dissected out immediately. On the following day these extracts were injected intervenously into a white rat. This rat showed a significant fall for a period of several hours in the production of CO<sub>2</sub> as compared to control values. Thus, we have a strong suggestion



that we are now on the track of an active agent which can suppress metabolism originating in the lungfish brain.

4. It is remarkable how rapidly the aestivating lungfish recovers his capacities for flight or fight. When the hole is first opened the fish appears to be extremely lethargic and in a state of somnolence. On disturbing the fish, however, he appears to be able to wake up extremely rapidly, and make attempts to bite or escape. It was not expected that the fish would react in this fashion.

5. On the basis of our proposed focus of this study onto hormones originating from the brain a request was made of our African agent to find some naturally aestivating lungfish in Uganda, to kill them in the field, extract their brains, process it in acetone and send the brains only, to us. On Dec. 28th a package containing 7 brains was received. These brains had been in transit for 5 days. They were extracted the afternoon of the day they arrived and injected into two mice the following day. One extract was taken from all of the fore-brains, the other from the mid and hind-brains. Neither of the two test rats showed a depression in their oxygen consumption or  $CO_2$  output (our oxygen consumption apparatus has at last arrived and is in commission). This was disappointing, but since we did not know the exact history of these brains, we can not interpret this finding until this information has been received. A delay of 5-7 days may, and indeed probably does, result in degradation of activity into the brain.

6. At the present time the two remaining large fish have

been in aestivation for periods of 5 weeks and 3 weeks respectively and next week it is planned to move both fish from the mud rapidly chop off the head, dissect the brain and perform the extracts. The testing of these extracts on rats is planned for January 22nd.

7. With the experience behind us, as mentioned above, we wish to pursue further obtaining of brain material from naturally aestivating fish in Uganda. There may be inherent difficulties in this procedure, however, due to inability to kill the fish rapidly, struggling of the fish under field conditions, the deterioration of the brain in hot weather following death and before extraction, and the several days it takes in transit from Uganda to Denver. Therefore, we wish to continue to import a few large fish and to force aestivation ourselves under controlled laboratory conditions.

We are very encouraged by these results and look forward with great impatience to our next studies.

Very respectfully submitted,

Henry Swan, M.D.  
Principal Investigator

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